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Short Latency Afferent Inhibition: Effects of Ageing

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Non-invasive techniques for testing the integrity of cholinergic networks in patients with neurodegenerative diseases are of increasing interest clinically. Short-latency afferent inhibition (SAI) is one example. SAI is a paired-pulse transcranial magnetic stimulation (TMS) technique in which TMS motor evoked potentials (MEPs) recorded in hand muscles are attenuated by conditioning stimuli to a peripheral nerve 20ms before the magnetic stimulus to the motor cortex (Tokimura et al. , 2000, Chen et al. , 2008, Chen, 2013).

Although the precise circuitry mediating SAI is unknown, it is likely to involve a fast pathway from the periphery to the motor cortex via thalamocortical projections, either directly or indirectly (Chen, 2013). SAI is reduced in cholinergically-mediated dementias (Di Lazzaro et al. , 2002, Di Lazzaro et al. , 2005a, Di Lazzaro et al. , 2007b, Nardone et al. , 2008) and after administration of the muscarinic antagonist scopolamine (Di Lazzaro et al. , 2000); it is also increased after a single dose of a cholinesterase inhibitor and may predict long term response to this drug (Di Lazzaro et al. , 2005a). This has led to interest in this neurophysiological technique as a biomarker for cognitive decline in neurodegenerative diseases such as Alzheimer's and Lewy body diseases (Di Lazzaro et al. , 2002, Di Lazzaro et al. , 2007b, Nardone et al. , 2008, Celebi et al. , 2012, Yarnall et al. , 2013, Cromarty et al. , 2015). Other neurotransmitters that are involved in SAI include γ-aminobutyric acid (GABA) and dopamine, with pharmacological studies demonstrating differing effects on SAI with administration of benzodiazepines and L-DOPA/D2-agonists (Di Lazzaro et al. , 2005b, Di Lazzaro et al. , 2007a, Martorana et al. , 2009, Martorana et al. , 2013).

The decline in cholinergic and dopaminergic function and changes in sensorimotor integration during the ageing process might suggest that SAI should decrease with age. However, the small studies that have looked at the effects of age on SAI have produced conflicting results (Oliviero et al.

, 2006, Degardin et al. , 2011, Young-Bernier et al. , 2012). The aim of our study was to resolve this issue by testing SAI across a range of ages in a much larger sample of healthy controls.

Sixty-nine healthy participants were recruited from two cohorts: 43 from the ICICLE-PD study (Khoo et al. , 2013, Yarnall et al. , 2013, Yarnall et al. , 2014); 26 were students/staff at Newcastle University. Mean age was 53.4 years (standard deviation 21.7; range 21-90). Exclusion criteria were: (1) contraindications to magnetic stimulation; and (2) medication or medical conditions that could affect somatosensory evoked potentials (SEPs)/MEPs/SAI (Yarnall et al. , 2013). All experiments were approved by a local research ethics committee and performed according to the Declaration of Helsinki, with subjects providing written informed consent.

Surface electromyogram (EMG) was recorded from abductor pollicis brevis (APB) and first dorsal interosseous (FDI) of the dominant upper limb with adhesive Ag-AgCI gel electrodes (Biosense Medical Ltd). SEPs were recorded via adhesive EEG electrodes (Neuroline 720, Ambu, Denmark) applied to the scalp using a bipolar montage (F3-C3 or F4-C4; international 10-20 system).

Signals were amplified (EMG gain 1000-2000; EEG gain 50k) and bandpass filtered (EMG 30 Hz-2kHz; EEG 3Hz-2kHz), using a Digitimer D360 system (Letchworth Garden City, Herts, UK), before being digitised at 5kHz by a Power1401 interface (Cambridge Electronic Design Ltd, Cambridge, UK) connected to a computer running Spike2 software (Cambridge Electronic Design Ltd).

SEPs were obtained by stimulating the median nerve at the wrist and averaging evoked EEG responses (2000 raw sweeps; repetition rate 5Hz). Stimuli (single pulses; pulse width 200µs; range 4 to 25 mA) were delivered using a constant current stimulator (Digitimer DS7A) via adhesive electrodes (cathode proximal; Biosense Medical Ltd). Stimulus intensity was adjusted to just above motor threshold, as determined by a visible twitch in APB.

TMS of the motor cortex was performed using a high power Magstim 200 (Magstim Co. Whitland, Dyfed, Wales) circular TMS coil (130mm diameter). An anticlockwise coil current was used to stimulate the left hemisphere (right hand) and vice versa. MEPs were recorded from the

contralateral FDI muscle. Resting motor threshold was determined as the percentage of maximum stimulator output which elicited a threshold MEP (approximately 50μV in 5 out of 10 trials) at rest. The SAI protocol used was based on Tokimura et al.'s study (Tokimura et al. , 2000) and described in detail elsewhere (Yarnall et al. , 2013, Cromarty et al. , 2015). In short, conditioning electrical stimuli were delivered to the median nerve and test stimuli to the motor cortex at five interstimulus intervals (ISIs) relative to the latency of the N20 component of the SEP to median nerve stimulation (from N20 to N20+4ms in 1ms steps). The sequence of unconditioned and conditioned MEPs at each ISI was randomized during testing. Peak-to-peak amplitude of conditioned MEPs at each ISI were averaged and expressed as a percentage of the averaged unconditioned MEP (Di Lazzaro et al. , 2000, Nardone et al. , 2008). Normalisation of the test stimulus is essential in comparative studies. For the test MEP this should ideally be set in the middle of the stimulus-response curve for each subject, thus avoiding ceiling or floor effects. However, deriving MEP recruitment curves is time-consuming. Alternative, more rapid methods have therefore been used for afferent inhibition studies. One approach is to set the test MEP peak-to-peak amplitude to approximately 1mV (Di Lazzaro et al. , 2000). The other is to set the magnetic stimulator at a predefined level above resting motor threshold (Denoordhout et al. , 1992). Recent evidence has shown that the latter is the less variable method for making between subject comparisons (Pitcher et al. , 2015). We therefore set the magnetic test stimulus at 20% above resting motor threshold for each subject.

Examples of MEPs acquired in our SAI experiments from a 23-and a 66-year old are shown in Figure 1A and 1B, respectively. SAI across the entire group was 61.7 ± 21.7% and conditioned responses ranged from 14.3% to 128.6% of control (Figure 1C). No significant correlation was seen between age and SAI (r= -0.084, p=0.495). There was also no correlation between MEP amplitude and SAI (r=0.103, p=0.401), even when controlling for age (r=0.053, p=0.666) ~~confirming that the size of the test MEP amplitude had no effect on SAI~~. General clinical and neurophysiological characteristics of participants by decade are demonstrated in Supplementary Table 1. Overall, RMT, MEP and median nerve stimulation decreased with age, whilst N20 amplitude increased.

This is the largest study to date to examine the effect of age on SAI in healthy, cognitively normal participants. In agreement with two small studies (Oliviero et al. , 2006, Degardin et al. , 2011), with sample sizes of 28 and 42, respectively, but in contrast to a more recent larger (n=56) study (Young-Bernier et al. , 2012), we found that SAI did not correlate with age and hence can be considered a robust phenomenon across all age groups. In keeping with work from others, mean MEP amplitude and RMT were significantly smaller in older than younger participants (Oliviero et al. , 2006, Silbert et al. , 2006, Young-Bernier et al. , 2012), with greater N20 amplitude as age increased (Hagiwara et al. , 2014).

One advantage of our dataset is that we included more participants in the older age-group (n=39; range 60-90) compared to previous studies (Oliviero et al. , 2006, Degardin et al. , 2011), which included 31 (age-range 65-82; mean=70), 14 (age-range 51-74; mean=62) and 22 (age-range not stated; mean=71) older participants respectively. Moreover, in the study by Young-Bernier et al. where SAI was significantly reduced in the older compared to younger age group, almost half of the senior group exhibited low levels of inhibition, and several exhibited facilitation (average conditioned MEP ≥ 100%) (Young-Bernier et al. , 2012). Only two subjects in our group demonstrated facilitation; one was aged 38 (conditioned MEP 107.2%) and one aged 77 (MEP 128.6%).

~~A potential limitation of this study is that certain age groups within this study had small participant numbers, which may limit the ability to detect age dependent effects.~~

A potential limitation of our study is that certain age groups had small participant numbers, which may limit the ability to detect age-dependent effects. Moreover, whilst there was no statistical correlation between SAI and MEP amplitude, given the evidence that SAI is reduced with increasing test MEP amplitudes (Udupa et al. , 2009, Ni et al. , 2011), we cannot entirely exclude the possibility that age-dependent changes might have been masked by the wide variation in test MEP amplitude in our cohort.

SAI has potential as a biomarker of cholinergic dysfunction in patients with neurodegenerative diseases (Di Lazzaro et al. , 2002, Di Lazzaro et al. , 2005a, Di Lazzaro et al. , 2007b, Nardone et al. , 2008, Celebi et al. , 2012, Rochester et al. , 2012, Yarnall et al. , 2013). With larger normal datasets and standardisation of the technique, it should be possible to establish SAI as an electrodiagnostic test for use in older patient groups with overt or sub-clinical neurodegenerative disease. Furthermore, our observation that SAI is a robust phenomenon unaffected by ageing suggests that it should be possible to extrapolate the results of SAI experiments in younger healthy controls.

Figure Legend

Figure 1

A & B. Examples of short-latency afferent inhibition (SAI) in healthy individuals aged 23 (***A***), at an interstimulus interval (ISI) of N20+1ms, and aged 66 (***B***), at an ISI of N20+3ms. Unconditioned (grey) and conditioned (black) motor evoked potentials (MEPs) recorded from *first dorsal interosseous* (FDI) have been aligned to the cortical stimulus (black arrow) and superimposed for comparison. Each trace represents an average of 20 raw MEPs. ***C.*** **Group data.** Box plots for each decade (median, interquartile range, with whiskers representing the highest and lowest values that are not outliers). Numbers in each sample are shown in brackets above each box. The solid horizontal line shows mean SAI for the whole sample (n=69); the grey box demarcates 2 standard deviations of the mean.

References

Celebi O, Temucin CM, Elibol B, Saka E. Short latency afferent inhibition in Parkinson's disease patients with dementia. Mov Disord. 2012;27:1052-5.

Chen R. Biomarker for Mild Cognitive Impairment: Is Short Latency Afferent Inhibition the Answer? Mov Disord. 2013;28:1171-2.

Chen R, Cros D, Curra A, Di Lazzaro V, Lefaucheur JP, Magistris MR, et al. The clinical diagnostic utility of transcranial magnetic stimulation: Report of an IFCN committee. Clin Neurophysiol. 2008;119:504-32.

Cromarty RA, Elder GJ, Graziadio S, Baker M, Bonanni L, Onofrj M, et al. Neurophysiological biomarkers for Lewy body dementias. Clin Neurophysiol. 2015;10.1016/j.clinph.2015.06.020.

Degardin A, Devos D, Cassim F, Bourriez J-L, Defebvre L, Derambure P, et al. Deficit of sensorimotor integration in normal aging. Neurosci Lett. 2011;498:208-12.

Denoordhout AM, Rothwell JC, Day BL, Dressler D, Nakashima K, Thompson PD, et al. Effect of Digital Nerve Stimuli on Responses to Electrical or Magnetic Stimulation of the Human Brain. J Physiol (Lond). 1992;447:535-48.

Di Lazzaro V, Oliviero A, Pilato F, Saturno E, Dileone M, Marra C, et al. Neurophysiological predictors of long term response to AChE inhibitors in AD patients. Journal of neurology, neurosurgery, and psychiatry. 2005a;76:1064-9.

Di Lazzaro V, Oliviero A, Profice P, Pennisi MA, Di Giovanni S, Zito G, et al. Muscarinic receptor blockade has differential effects on the excitability of intracortical circuits in the human motor cortex. Exp Brain Res. 2000;135:455-61.

Di Lazzaro V, Oliviero A, Tonali PA, Marra C, Daniele A, Profice P, et al. Noninvasive in vivo assessment of cholinergic cortical circuits in AD using transcranial magnetic stimulation. Neurology. 2002;59:392-7.

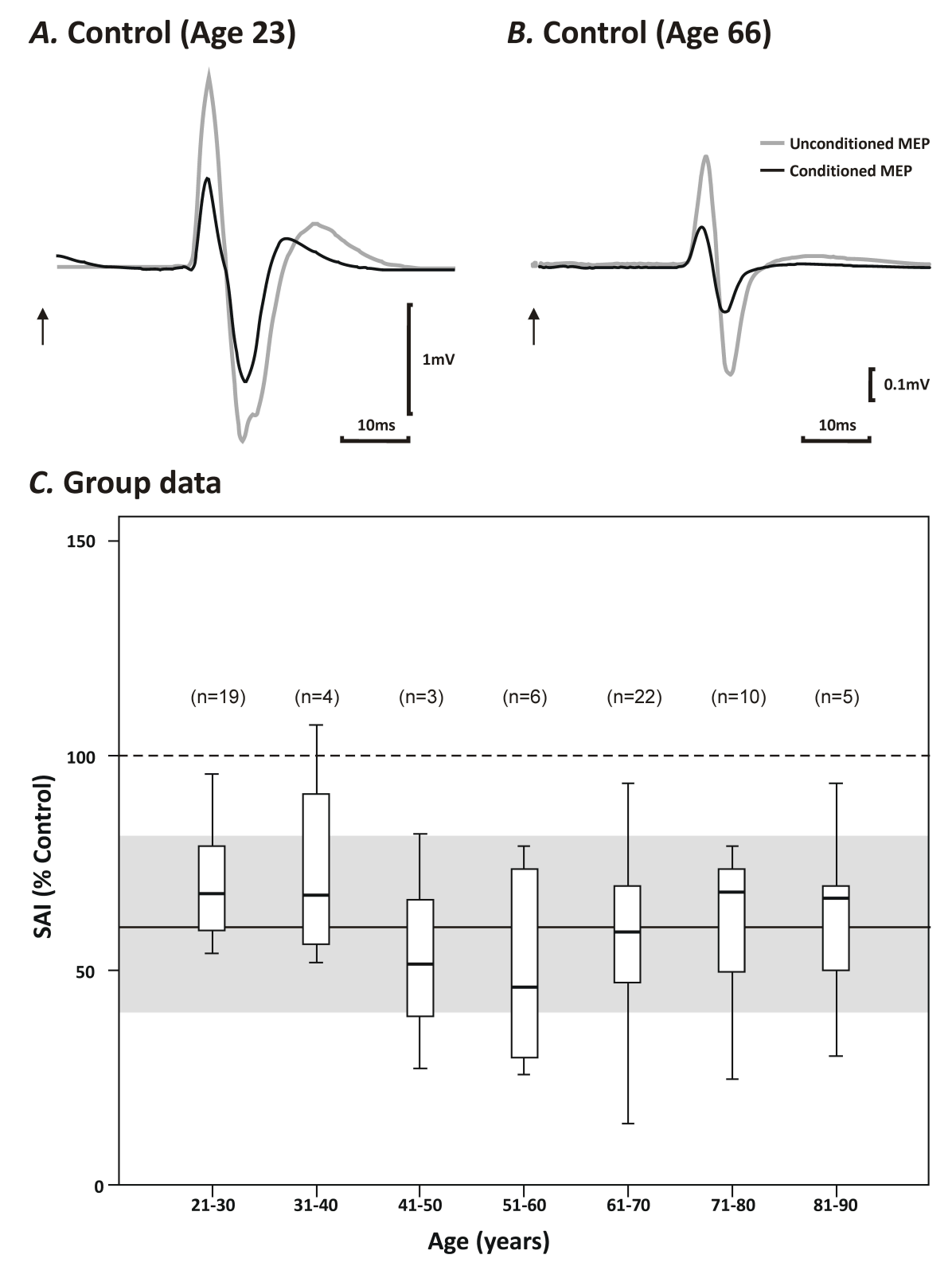
Di Lazzaro V, Pilato F, Dileone M, Profice P, Ranieri F, Ricci V, et al. Segregating two inhibitory circuits in human motor cortex at the level of GABAA receptor subtypes: a TMS study. Clin Neurophysiol. 2007a;118:2207-14.

Di Lazzaro V, Pilato F, Dileone M, Saturno E, Profice P, Marra C, et al. Functional evaluation of cerebral cortex in dementia with Lewy bodies. Neuroimage. 2007b;37:422-9.

Di Lazzaro V, Pilato F, Dileone M, Tonali PA, Ziemann U. Dissociated effects of diazepam and lorazepam on short-latency afferent inhibition. The Journal of physiology. 2005b;569:315-23.

Hagiwara K, Ogata K, Okamoto T, Uehara T, Hironaga N, Shigeto H, et al. Age-related changes across the primary and secondary somatosensory areas: An analysis of neuromagnetic oscillatory activities. Clin Neurophysiol. 2014;125:1021-9.

Khoo TK, Yarnall AJ, Duncan GW, Coleman S, O'Brien J, Brooks D, et al. The spectrum of nonmotor symptoms in early Parkinson disease. Neurology. 2013;80:1-6.
Martorana A, Di Lorenzo F, Esposito Z, Lo Giudice T, Bernardi G, Caltagirone C, et al. Dopamine D-2-agonist Rotigotine effects on cortical excitability and central cholinergic transmission in Alzheimer's disease patients. Neuropharmacology. 2013;64:108-13.
Martorana A, Mori F, Esposito Z, Kusayanagi H, Monteleone F, Codeca C, et al. Dopamine modulates cholinergic cortical excitability in Alzheimer's disease patients. Neuropsychopharmacology. 2009;34:2323-8.
Nardone R, Bergmann J, Kronbichler M, Kunz A, Klein S, Caleri F, et al. Abnormal short latency afferent inhibition in early Alzheimer's disease: a transcranial magnetic demonstration. Journal of Neural Transmission. 2008;115:1557-62.
Ni Z, Charab S, Gunraj C, Nelson AJ, Udupa K, Yeh IJ, et al. Transcranial Magnetic Stimulation in Different Current Directions Activates Separate Cortical Circuits. J Neurophysiol. 2011;105:749-56.
Oliviero A, Profice P, Tonali PA, Pilato F, Saturno E, Dileone M, et al. Effects of aging on motor cortex excitability. Neurosci Res. 2006;55:74-7.
Pitcher JB, Doeltgen SH, Goldsworthy MR, Schneider LA, Vallence AM, Smith AE, et al. A comparison of two methods for estimating 50% of the maximal motor evoked potential. Clin Neurophysiol. 2015;126:2337-41.
Rochester L, Yarnall AJ, Baker MR, David RV, Lord S, Galna B, et al. Cholinergic dysfunction contributes to gait disturbance in early Parkinson's disease. Brain. 2012;135:2779-88.
Silbert LC, Nelson C, Holman S, Eaton R, Oken BS, Lou JS, et al. Cortical excitability and age-related volumetric MRI changes. Clin Neurophysiol. 2006;117:1029-36.
Tokimura H, Di Lazzaro V, Tokimura Y, Oliviero A, Profice P, Insola A, et al. Short latency inhibition of human hand motor cortex by somatosensory input from the hand. The Journal of physiology. 2000;523 Pt 2:503-13.
Udupa K, Ni Z, Gunraj C, Chen R. Interactions between short latency afferent inhibition and long interval intracortical inhibition. Exp Brain Res. 2009;199:177-83.
Yarnall AJ, Breen DP, Duncan GW, Khoo TK, Coleman S, Firbank MJ, et al. Characterizing Mild Cognitive Impairment in Incident Parkinson's Disease: The ICICLE-PD Study Neurology. 2014;82:1-9.
Yarnall AJ, Rochester L, Baker MR, David R, Khoo TK, Duncan GW, et al. Short latency afferent inhibition: a biomarker for mild cognitive impairment in Parkinson's disease? Mov Disord. 2013;28:1285-8.
Young-Bernier M, Davidson PSR, Tremblay F. Paired-pulse afferent modulation of TMS responses reveals a selective decrease in short latency afferent inhibition with age. Neurobiol Aging. 2012;33:835.e1-11.


A. Control (Age 23)
B. Control (Age 66)
Unconditioned MEP
Conditioned MEP
1mV
10ms
0.1mV
10ms
C. Group data
150
100
50
0
SAI (% Control)
(n=19)
(n=4)
(n=3)
(n=6)
(n=22)
(n=10)
(n=5)
21-30
31-40
41-50
51-60
61-70
71-80
81-90
Age (years)